splitting in eq 1. A binuclear Ni(II) complex with such large exchange interaction is without precedence in inorganic chemistry and may be a consequence of a bridging thiolate. The synthesis and characterization of appropriate binuclear model compounds is required to evaluate this proposal.

Acknowledgment. This work was supported by grants from the National Science Foundation (DMB-8921986 to M.K.J.) and the U.S. Department of Agriculture (89-37120-4804 to D.E.W.).

Triplex Formation of Oligonucleotides Containing 2′-*O*-Methylpseudoisocytidine in Substitution for 2′-Deoxycytidine

Akira Ono, Paul O. P. Ts'o, and Lou-sing Kan*

Department of Biochemistry
School of Hygiene and Public Health
Johns Hopkins University
615 North Wolfe Street, Baltimore, Maryland 21205

Received August 20, 1990

Recently, studies of sequence-specific triplex formation of short synthetic oligonucleotides (or their analogues) have been of prime interest.[1-6] In this report, a synthesis of a new oligonucleotide analogue containing 2-amino-5-(2-*O*-methyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (2′-*O*-methylpseudoisocytidine or **1**) in substitution for 2′-deoxycytidine and the triplex formation of this oligonucleotide analogue with a target duplex at neutral pH are reported.

A triplex can be formed as a triad consisting of a homopyrimidine strand, a homopurine strand, and a homopyrimidine strand.[7-11] The third strand (i.e., the second pyrimidine strand) is located in the major groove of a duplex with Watson–Crick base pairing.[12-16] Thymines or cytosines in the third strand form Hoogsteen type hydrogen bondings with adenines or guanines in the homopurine strand, respectively. Since protonation of cytosine bases in acidic conditions is essential in order to provide the second hydrogen bonding between the protonated cytosine and guanine in the Hoogsteen pair of the triad (Figure 1, upper-left panel), this C-G-C^+ triad is unstable in physiological conditions.[17-19] This requirement limits the formation of triplex in living cells since the cellular pH is usually above pH 7.0.

To overcome this limitation, we designed and synthesized an oligonucleotide containing **1** which may form Hoogsteen type base pairings through hydrogen bondings with guanine in neutral and basic conditions (Figure 1, upper-right panel). As indicated in the figure, this nucleoside **1** already contains one hydrogen at the N-3 position for hydrogen bonding with the guanine in the Hoogsteen pair of the triad. Also, methoxy substitution at 2′-position of pyrimidine nucleosides in a third strand stabilized the triplex formation.[20]

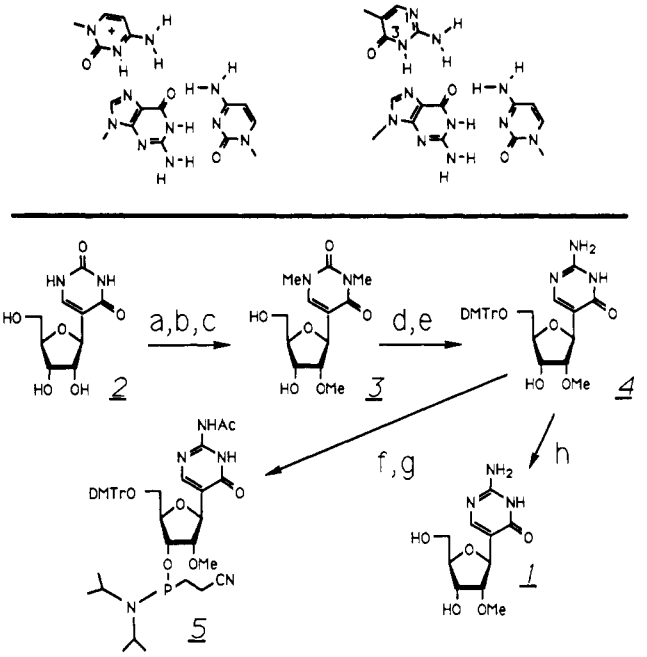

Figure 1. The hydrogen-bonding schemes of triplexes of CGC^+ (upper left) and CG**1** (upper right). The lower panel is a scheme for the synthesis of **1** and **5**: **2** was obtained from Kyowa Hakko, U.S.A., Inc. (New York, NY). (a) Protection of the 3′ and 5′ hydroxyl groups according to Pankiewicz et al.[28] (b) Methylation of the 2′-hydroxyl group; iodomethane (40 equiv), Ag_2O (10 equiv), 25 °C, 7 days.[22] (c) Deprotection of the silyl group; Bu_4NF (2.5 equiv), THF, 25 °C, 1 h; 58% from the 3′- and 5′-protected compound. (d) Protection of the 5′-hydroxyl group; dimethoxytrityl (DMT) chloride (1.05 equiv), pyridine, 25 °C, 12 h; 94%. (e) Conversion of the base moiety; guanidine (100 equiv), sodium ethoxide (70 equiv), absolute ethanol, reflux for 1 h;[29] 61%. The α-anomer was not detected. (f) Protection of the amino group; acetic anhydride, DMF, 25 °C, 12 h, 78%. (g) (2-Cyanoethyl)-*N*,*N*-diisopropylphosphonamidous chloride (1.5 equiv),[30] $EtiPr_2N$ (2 equiv), CH_2Cl_2, 25 °C, 30 min; 71%. (h) Acetic acid (80%), 25 °C, 12 h; 78%.

A scheme for the synthesis of **1**[21] and its amidite synthon **5** is shown in the lower panel of Figure 1. An octamer 5′-(TT1TT1TT)3′ (**a**) was synthesized on a DNA synthesizer (Applied Biosystem). After deblocking and purification, the oligomer a of this preparation showed one sharp peak by HPLC analysis. After hydrolysis of the oligomers by snake venom phosphodiesterase and alkaline phosphatase to nucleosides, the nucleoside composition for each oligomer was confirmed by HPLC.

We studied a triplex formation of the oligomer **a** with an undecameric target duplex 5′d(AAGAAGAAGAA)3′–5′d-(TTCTTCTTCTT)3′ (**d**). Both 5′d(TTCTTCTT)3′ (**b**) and 5′(TTCmTTCmTT)3′ (**c**, Cm = 2′-*O*-methylcytidine[22]) were used as controls for the third strand. An octamer **a** or **b** or **c** was mixed with the duplex **d** in a buffer, and the thermally induced transitions of the helices in each mixture was studied by measurement of the UV absorption at 260 nm at pH 7 (Figure 2, upper panel). Both the duplex **d** alone and the mixtures consisting of **b** and **d** or of **c** and **d** showed only one transition (T_m = 42 °C, Figure 2, lower panel) which was attributable to the melting of the duplex **d** itself. On the other hand, the mixture of **a** and **d** showed two transitions

(1) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645–650.
(2) François, J.-C.; Saison-Behmoaras, T.; Barbier, C.; Chassignol, M.; Thuong, N. T.; Hélène, C. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9702–9706.
(3) Maher, L. J., III; Wold, B.; Dervan, P. B. *Science* **1989**, *245*, 725–730.
(4) Hanvey, J. C.; Shimizu, M.; Wells, R. D. *Nucleic Acids Res.* **1990**, *18*, 157–161.
(5) François, J.-C.; Saison-Behmoaras, T.; Thuong, N. T.; Hélène, C. *Biochemistry* **1989**, *28*, 9617–9619.
(6) Cooney, M.; Czernuszewicz, G.; Postel, E. H.; Flint, S. J.; Hogan, M. E. *Science* **1988**, *241*, 456–459.
(7) Felsenfeld, G.; Davies, D. R.; Rich, A. *J. Am. Chem. Soc.* **1957**, *79*, 2023–2024.
(8) Felsenfeld, G.; Miles, T. *Annu. Rev. Biochem.* **1967**, *36*, 407–448.
(9) Michelson, A. M.; Massoulié, J.; Guschlbauer, W. *Prog. Nucleic Acid Res. Mol. Biol.* **1967**, *6*, 83–141.
(10) Letai, A. G.; Palladino, M. A.; Fromm, E.; Rizzo, V.; Fresco, J. R. *Biochemistry* **1988**, *27*, 9108–9112.
(11) Lee, J. S.; Woodsworth, M. L.; Latimer, L. J. P.; Morgan, A. R. *Nucleic Acids Res.* **1984**, *12*, 6603–6614.
(12) Arnott, S.; Bond, P. J.; Selsing, E.; Smith, P. J. C. *Nucleic Acids Res.* **1976**, *3*, 2459–2470.
(13) des los Santos, C.; Rosen, M.; Patel, D. *Biochemistry* **1989**, *28*, 7282–7289.
(14) Rajagopal, P.; Feigon, J. *Nature* **1989**, *339*, 637–640.
(15) Rajagopal, P.; Feigon, J. *Biochemistry* **1989**, *28*, 7859–7870.
(16) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(17) Lipsett, M. N. *Biochem. Biophys. Res. Commun.* **1963**, *11*, 224–228.
(18) Howard, F. B.; Frazier, J.; Lipsett, M. N.; Miles, H. T. *Biochem. Biophys. Res. Commun.* **1964**, *17*, 93–102.
(19) Thiele, D.; Guschlbauer, W. *Biopolymer* **1971**, *10*, 143–157.
(20) Unpublished data.
(21) Syntheses of **1** and **5** were monitored by NMR, UV, and CD methods.
(22) Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131–6148.

0002-7863/91/1513-4032$02.50/0

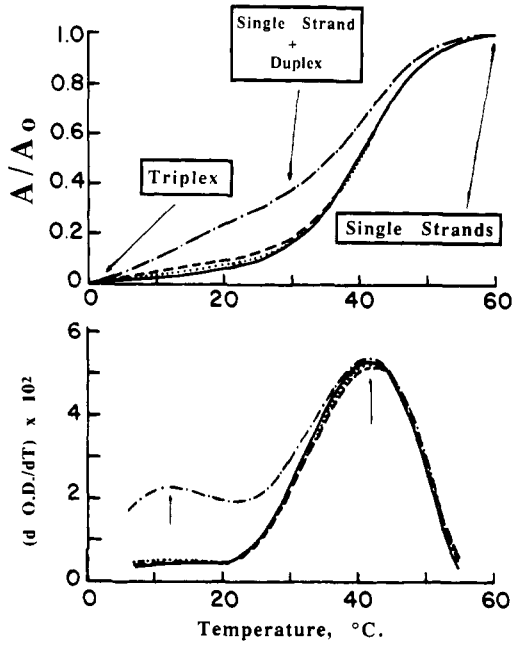

Figure 2. Upper panel: Relative absorbance, $A/A_0 = [A(T\,°C) - (A(0\,°C)]/[A(60\,°C) - A(0\,°C)]$, vs temperature at 260 nm. Lower panel: Absorbance change $[\Delta A(260\,nm)/\Delta T]$ vs temperature; (—) duplex d alone, (–·–) mixture of a and d, (···) mixture of b and d, (– – –) mixture of c and d. All solutions contain each oligomer (3 μM), 1.0 M NaCl, 20 mM $MgCl_2$, and 0.01 M sodium cacodylate, pH 7.0. T_m's are indicated by arrows.

(Figure 2, lower panel). One transition (T_m = 42 °C) was identical with the melting of **d** and the second transition (T_m = 12 °C) was surmised to be the dissociation of the third strand **a** from the duplex **d** as illustrated in the upper panel of Figure 2. This mixture of **a** and **d** showed similar transition profiles between pH 7 and pH 8.7 (data not shown).

Also, we studied the circular dichroism (CD) spectra of mixtures of oligomers. Spectra of a mixture of **a** and the duplex **d** at both 3 °C (dotted line) and 30 °C (solid line) in neutral condition are shown in Figure 3A. As a control, the sum of a spectrum of **a** and a spectrum of **d** in the same buffer at 3 °C is shown in the same figure (broken line). The control spectrum and the spectrum of the mixture at 30 °C showed similar patterns. In contrast, the spectrum of the mixture at 3 °C showed a different pattern, especially in the shorter wavelength region. A positive band at 215 nm in the control spectrum changed to a negative band in the spectrum of the mixture at 3 °C. This difference indicated the association of the third strand with the duplex.[23-26] Furthermore, a CD mixing study showed that the association has a 1:1 stoichiometry. These results indicated the triplex formation of **a** with **d**.

It has been reported recently that the substitution of 2'-deoxycytidine by 2'-deoxy-5-methylcytidine (^{m}C) and the substitution of thymidine by 2'-deoxy-5-bromouridine in an oligomer can cause triplex formation above neutral pH.[27] In such a situation, the substitution at position 5 of pyrimidines was able to add a stabilizing factor (nature unknown) for the triplex. Our approach is different. A triplex can be formed without the requirement of protonation if one designs an oligonucleotide containing **1**, a 2'-deoxycytidine analogue, which contains a hydrogen

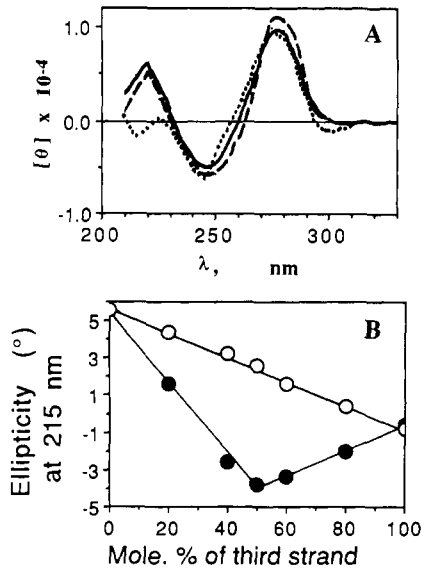

Figure 3. (A) CD spectra of mixtures of a and d at 3 °C (dotted line) and 30 °C (solid line) and the sum of a spectrum of a and a spectrum of the duplex d in the same buffer at 3 °C (broken line) with 3 μM a and d, respectively, in 1.0 M NaCl, 20 mM $MgCl_2$, 0.01 M sodium phosphate, pH 7.5. Molar ellipticity, $[\theta]$, is given per base residue. (B) CD mixing curves of a and d (filled circles) as well as b and d in 1.0 M NaCl, 20 mM $MgCl_2$, 0.01 M sodium phosphate, pH 7.5 at 3 °C (open circles).

at the N-3 (or N-1) position, and which can form a pair of hydrogen bonding with 2'-deoxyguanosine in the Hoogsteen scheme through this very hydrogen. With this innovative approach, it is anticipated that the possibility of triplex formation of an oligonucleotide analogue with genomic DNA in mammalian cells can be tested.

Acknowledgment. CD spectra were obtained by using a JASCO 500A CD spectrometer (in Dr. S. K. Yang's laboratory) at Uniformed Services University of Health Sciences. The work was supported in part by Department of Energy Grant DE-FG02-88 ER60636 and in part by National Cancer Institute Grant CA-42762-04A1. Finally, we thank Ms. T. Trapane for her helpful discussion.

(23) Lee, J. S.; Johnson, D. A.; Morgan, A. R. *Nucleic Acids Res.* **1979**, *6*, 3073-3091.
(24) Gray, D. M.; Morgan, A. R.; Ratliff, R. L. *Nucleic Acids Res.* **1978**, *5*, 3679-3695.
(25) Xodo, L. E.; Manzini, G.; Quadrifoglio, F. *Nucleic Acids Res.* **1990**, *18*, 3557-3564.
(26) Callahan, D. E.; Trapane, T. L.; Miller, P. S.; Ts'o, P. O. P.; Kan, L.-S. *Biochemistry* **1991**, *30*, 1650-1665.
(27) Povsic, T. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 3059-3061.
(28) Pankiewicz, K.; Matsuda, A.; Watanabe, K. A. *J. Org. Chem.* **1982**, *47*, 485-488.
(29) Hirota, K.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1978**, *43*, 1193-1197.
(30) Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843-5846.

Excited-State Properties in Supramolecular Systems. Luminescence and Intercomponent Interactions in a 3-Catenand and Some 3-Catenates

Nicola Armaroli,[1a] Vincenzo Balzani,*[1a]
Francesco Barigelletti,[1b] Luisa De Cola,[1a]
Jean-Pierre Sauvage,[1c] and Catherine Hemmert[1c]

Dipartimento di Chimica "G. Ciamician" dell'Università di Bologna 40126 Bologna, Italy
Istituto FRAE-CNR, 40126 Bologna, Italy
Laboratoire de Chimie Organo-Minerale, UA 422 au CNRS Institut de Chimie, Université Louis Pasteur 67000 Strasbourg, France

Received November 27, 1990
Revised Manuscript Received February 19, 1991

Supramolecular species obtained by assembling molecular components are currently the object of extensive photochemical

(1) (a) University of Bologna. (b) Istituto FRAE-CNR. (c) University of Strasbourg.

0002-7863/91/1513-4033$02.50/0